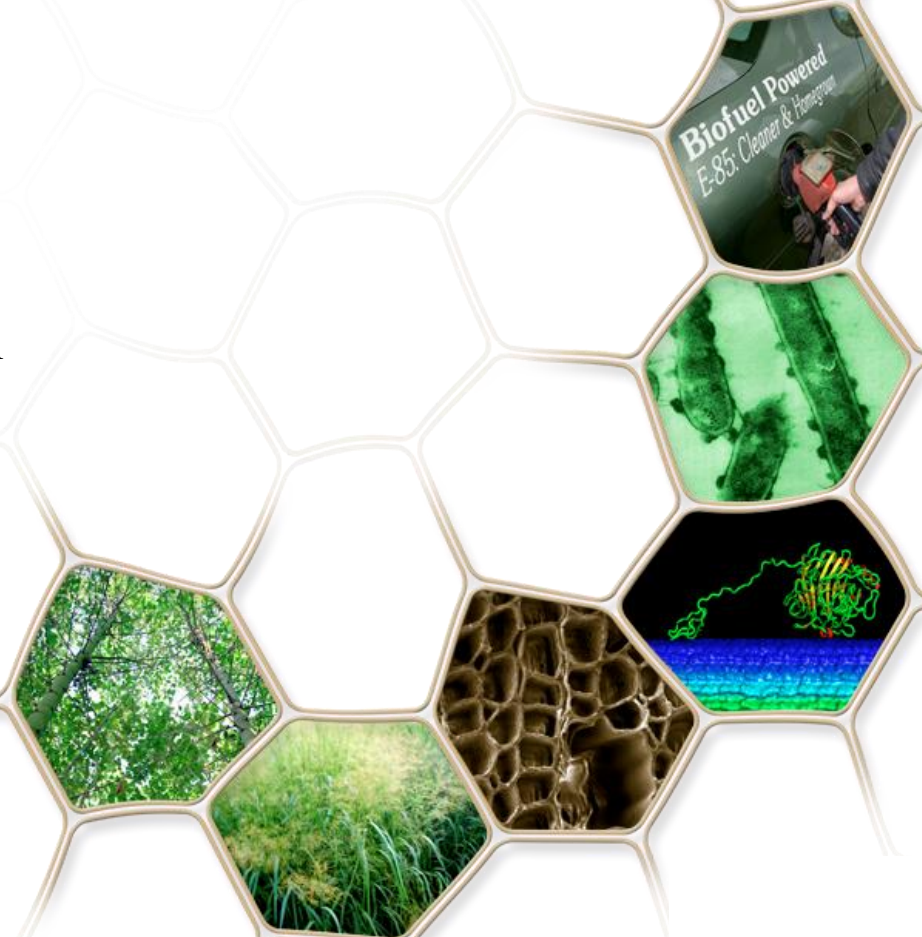


The Genetics of Carbon Allocation and Partitioning in *Populus*

Gerald A. Tuskan^{1,4}

Wellington Muchero¹, Priya Ranjan^{1,2},
Stephen DiFazio³, Tim Tschaplinski¹, Paul
Abraham^{1,2}, Jay Chen¹, Jeremy Schmutz⁵,
Dan Rokshar⁴, Udaya Kalluri¹, Nancy
Engle, and many others!!!

- 1 Oak Ridge National Laboratory, Oak Ridge, TN
- 2 University of Tennessee, Knoxville, TN
- 3 West Virginia University, Morgantown, WV
- 4 Joint Genome Institute, Walnut Creek, CA
- 5 HudsonAlpha, Huntsville, AL



Overview and Content of the Talk

- Carbon Allocation and Partitioning
- QTL Analyses
 - Carbon allocation
 - Carbon Partitioning
- GWAS Analyses
 - Aboveground Carbon Partitioning
- Host-driven Microbiome

Wallula QTL planting 1st year



Wallula QTL planting 2nd year



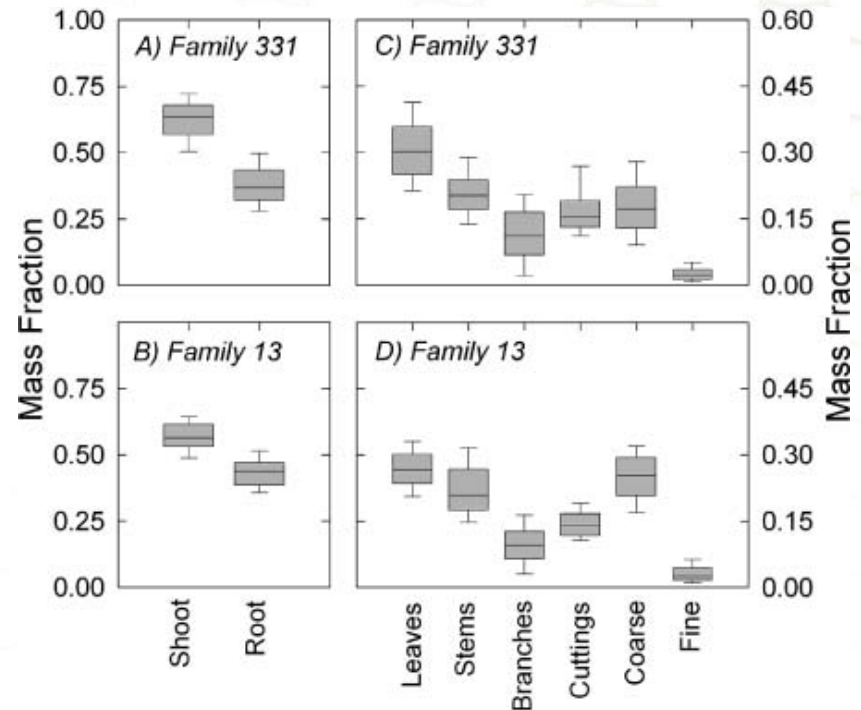
Carbon Allocation Above and Below Ground

- We destructively sampled two *Populus* pedigrees and segregated biomass into leaves, branches, stems, coarse roots and fine roots
- We used a saturated genetic map containing 800 SSR marker to locate QTLs associated with each biomass component
- Several highly significant QTLs were identified and interestingly there appears to be regions of the genome that independently control above and below ground traits.



Carbon Allocation Above and Below Ground

- We destructively sampled two *Populus* pedigrees and segregated biomass into leaves, branches, stems, coarse roots and fine roots
- We used a saturated genetic map containing 800 SSR marker to locate QTLs associated with each biomass component
- Several highly significant QTLs were identified and interestingly there appears to be regions of the genome that independently control above and below ground traits.

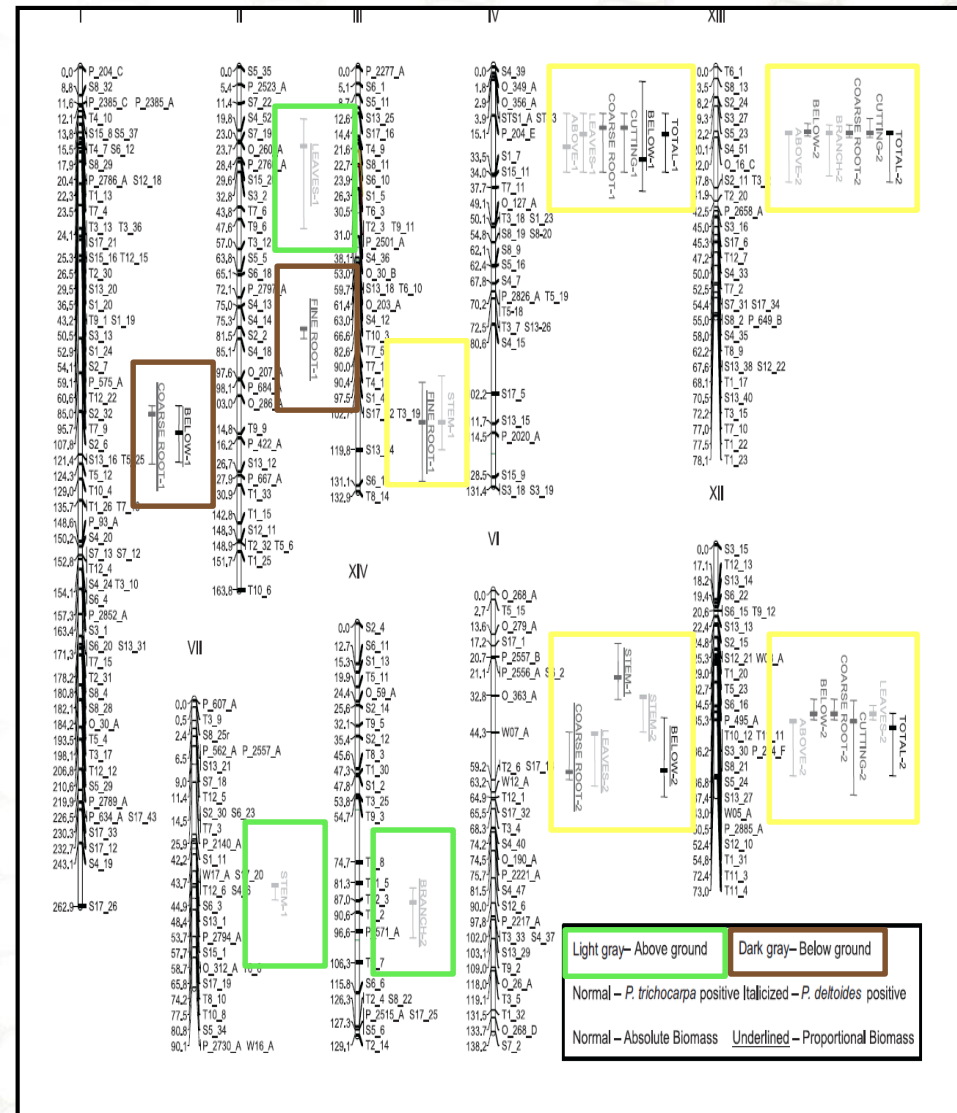


Carbon Allocation Above and Below Ground

- We destructively sampled two *Populus* pedigrees and segregated biomass into leaves, branches, stems, coarse roots and fine roots
- We used a saturated genetic map containing 800 SSR marker to locate QTLs associated with each biomass component
- Several highly significant QTLs were identified and interestingly there appears to be regions of the genome that independently control above and below ground traits.

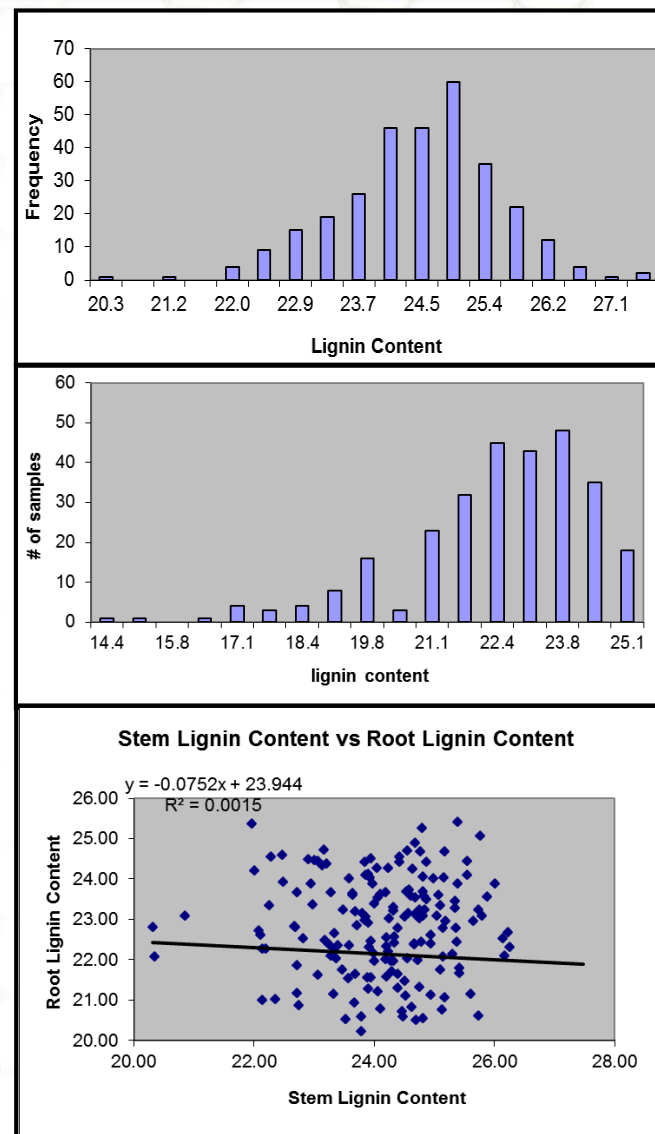
Wullschleger et al. (2005) Canadian J Forest Research 35:1779-1789.

Muchero et al. (2013) PloS ONE 8(1):e54468.



Carbon Partitioning QTL study

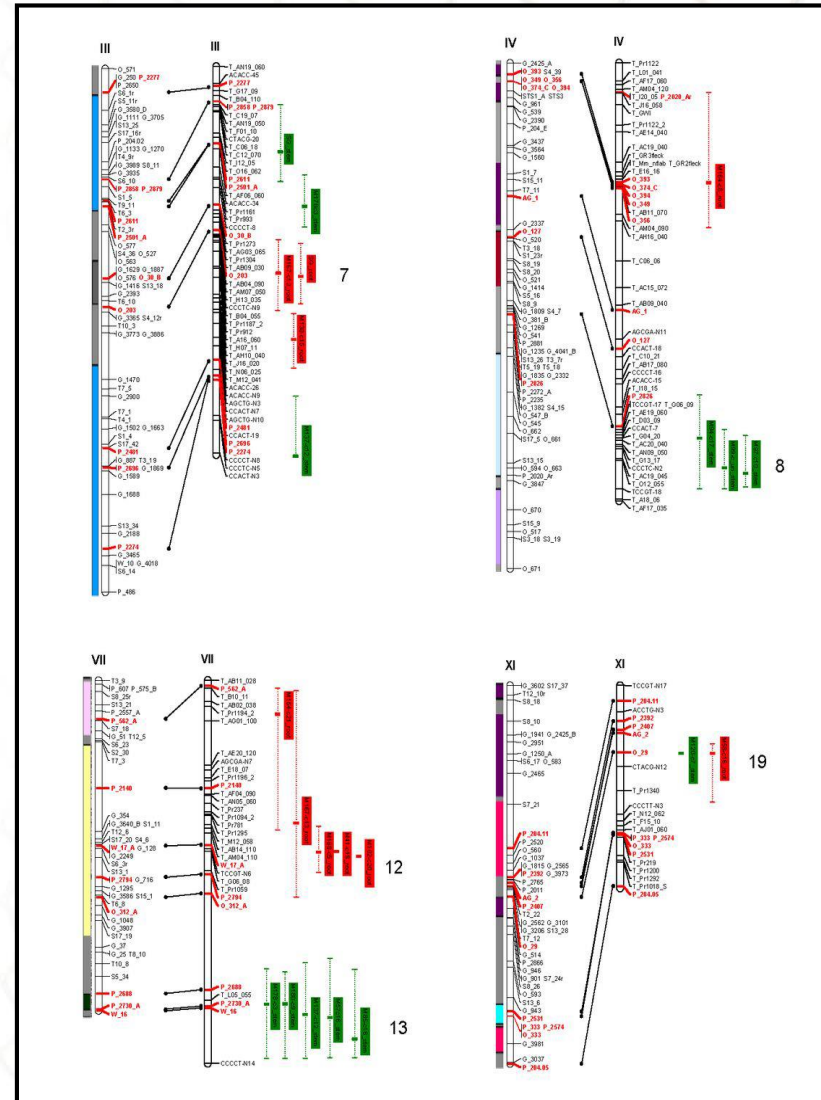
- We use MBMS pyrolysis to characterize cell wall chemistry in leaves, branches, stems, coarse roots and fine roots
- We again relied on the saturated genetic map containing 800 SSR marker to locate QTLs associated with each biomass component
- And again found that several highly significant QTLs were identified and interestingly there appears to be regions of the genome that independently control above and below ground traits.



- We use MBMS pyrolysis to characterize cell wall chemistry in leaves, branches, stems, coarse roots and fine roots
- We again relied on the saturated genetic map containing 800 SSR marker to locate QTLs associated with each biomass component
- And again found that several highly significant QTLs were identified and interestingly there appears to be regions of the genome that independently control above and below ground traits.

Yin et al. (2010) PLOS ONE 5(11):e14021.

Porth et al. (2013) New Phytologist 197:777-790.



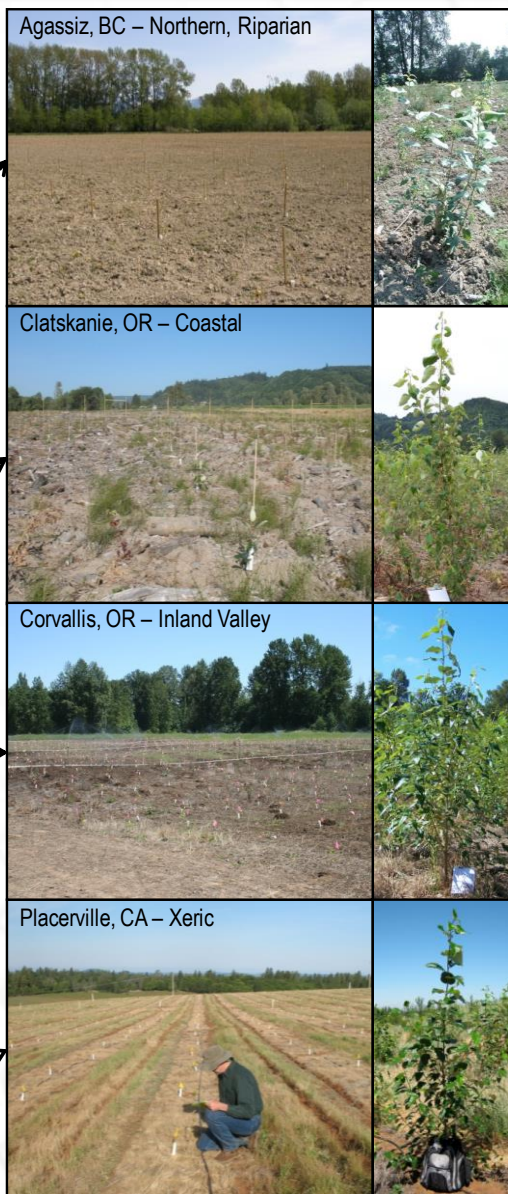
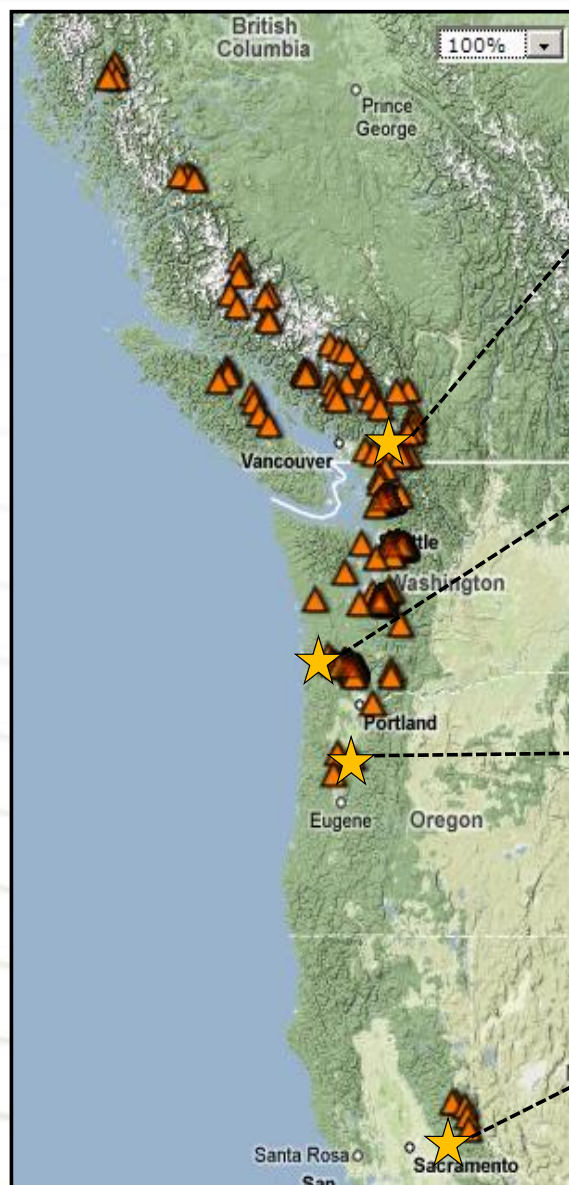
Populus Genome Released

- ***Populus* V3.0 assembly:**
 - Assembled with Arachne v20071016HA
 - Covers 423 Mb pairs out of 485 Mb total, an average read depth of 9.44X
 - Arranged in 1,446 scaffolds (2,585 gaps)
 - Integrates 81 Mb of finished sequence
 - Scaffold N50 (L50) = 8 (19.5 Mb)
 - Contig N50 (L50) = 206 (552.8 kb)
 - Represents ca. 97.3% of the genome
- ***Populus* V3.0 annotation:**
 - 75,566 RNAseq transcript assemblies
 - Constructed from 0.6 B pairs of paired-end Illumina RNAseq reads and 2.6 M 454-sequenced EST reads
 - 41,335 predicted gene models
 - 32% have splice variants
 - 73,013 total protein-coding transcripts
 - 90.5% of V2.2 loci were mapped to V3.0 loci



Tuskan et al. (2006) *Science*
196:726-737.

GWAS Population and SNP Detection



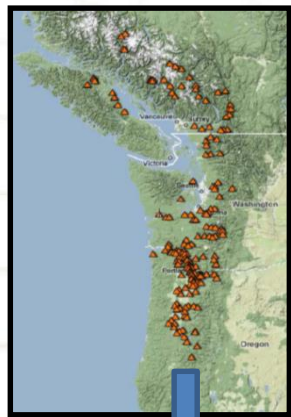
- 1084 unrelated genotypes clonally replicated in four contrasting environments
- Each genotype has been resequenced to a minimum 18X depth
- There are ca. 48 millions SNPs in the population, with a nucleotide variant every 10 bp

Slavov et al. (2012) *New Phytologist* 196:726-737.

Geraldes et al. (2013) *Molecular Ecology Resources* In press.

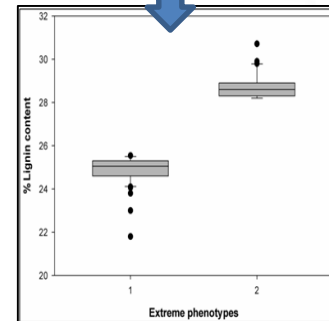
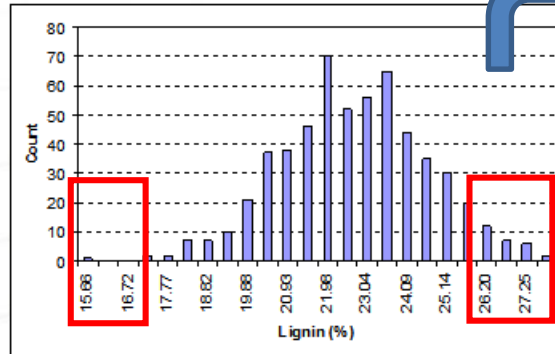
Variation in Lignin Composition and Content

Extreme variation contained in native populations of *Populus* detected in common garden experiments are linked to genes using Association Genetics



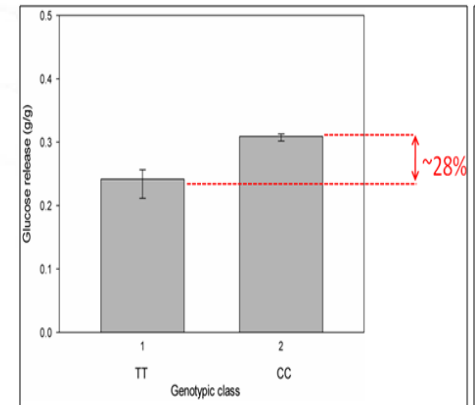
Assemble a population

Phenotype the population



Create a SNP library

GWAS Analysis



GWAS Carbon Allocation and Partitioning

- *Populus* stems vary in their roughness, from smooth to coarse
- This variation is linked to ecological function
- *Populus* roots also vary in this trait, and variation does not seem to be correlated
- The “corkiness” of the root bark may partially determine the rate of carbon turnover in soils

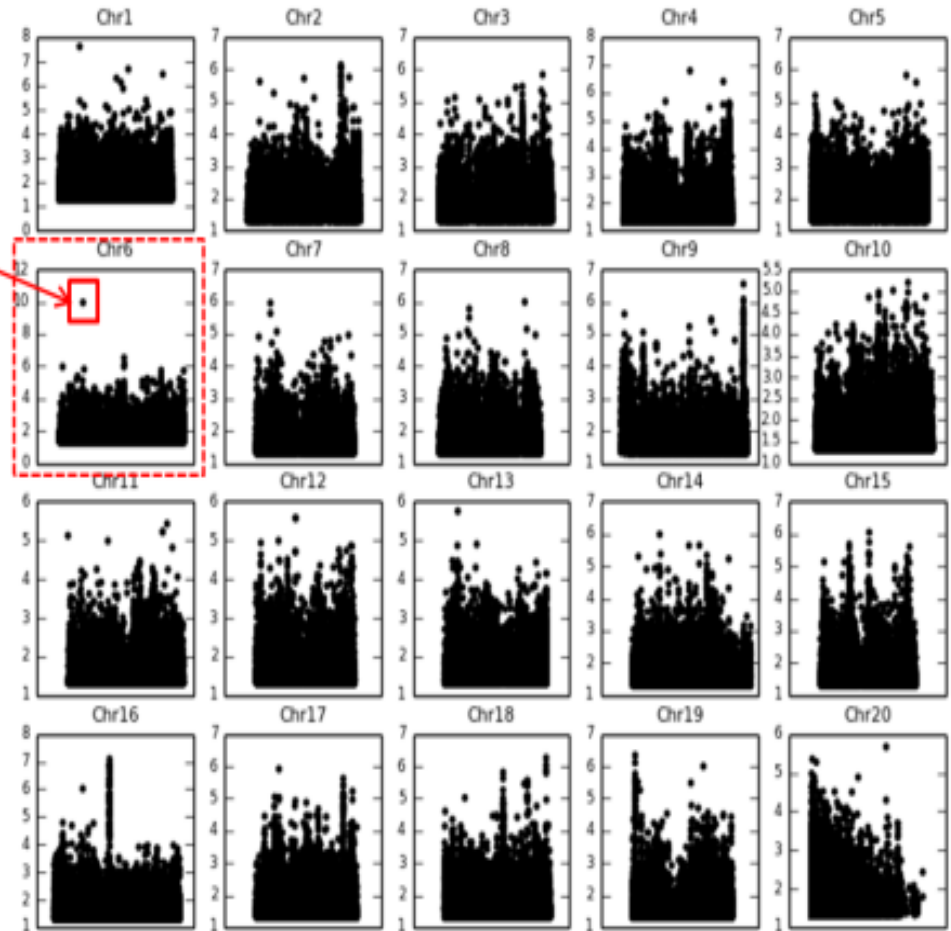


Jansson et al. (2010) BioSciences
60:685-696.

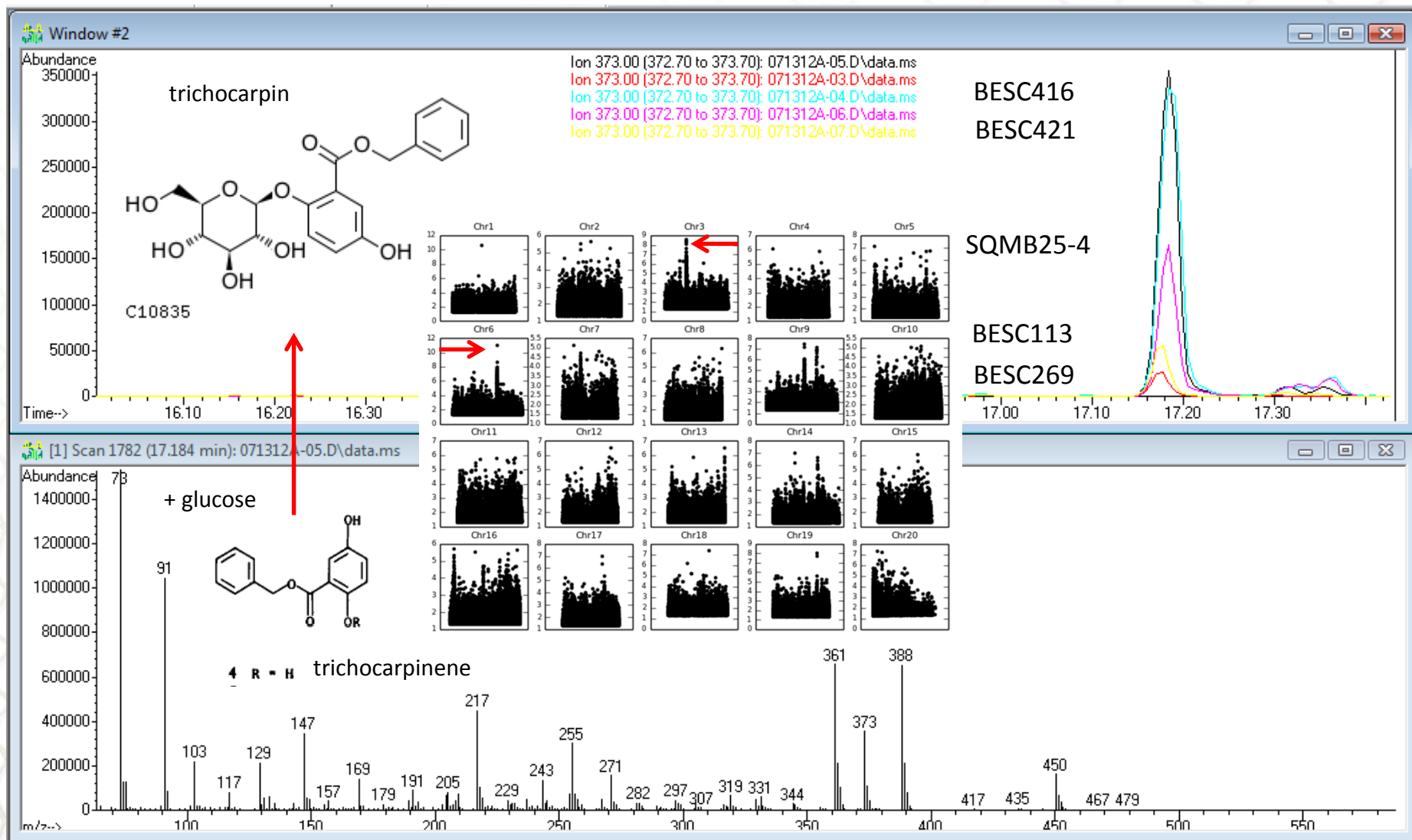
GWAS Results for Carbon Allocation



CONNEXIN: Encodes a receptor-like cytoplasmic kinase that acts as a spatial inhibitor of cell separation



GWAS Carbon Partitioning



Chr 6 [Potri.006G171200 - UDP-glucosyl transferase 78D2] (*confirms major mQTL)

Chr 3 [Potri.003G049600 - Plant PDR ABC transporter associated]

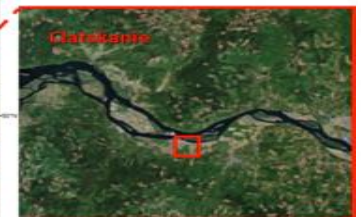
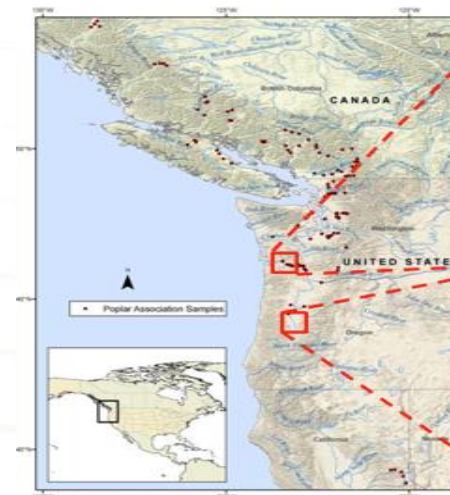
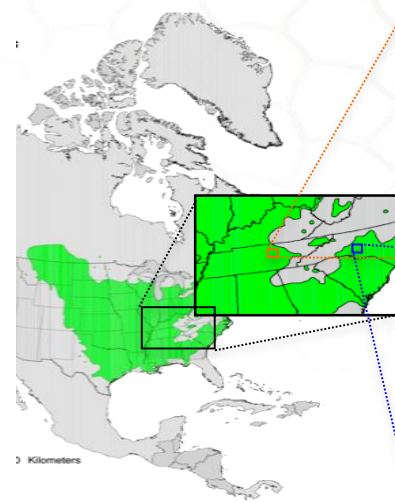
The Host Microbiome – Collection sites



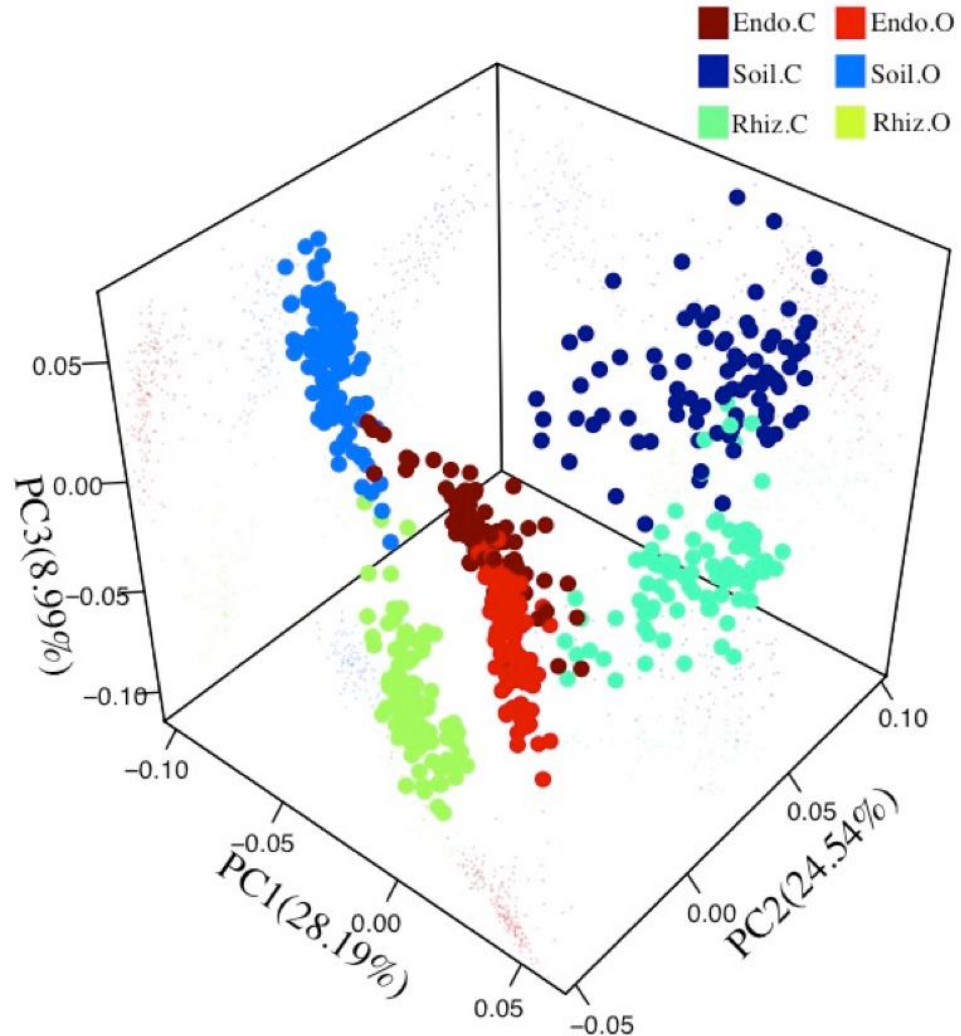
13 genotypes
Caney Fork
river, TN
11 genotypes
Yadkin river,
NC



1087 GWAS
population at 2-
locations



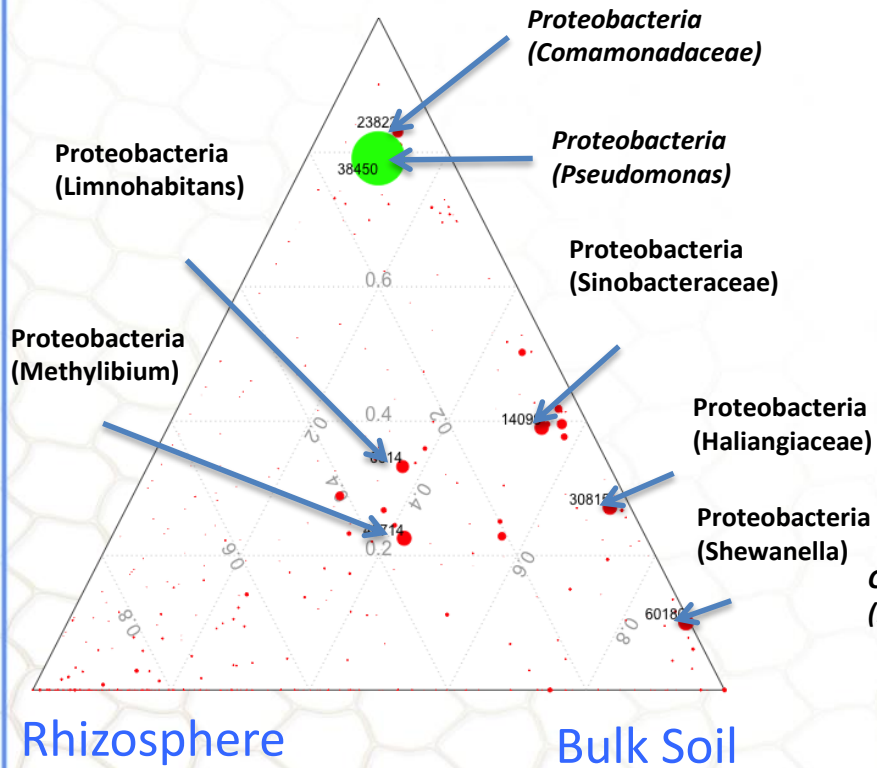
The Host Microbiome – Genotype vs. Environment



The Host Microbiome – The Microbial Atlas

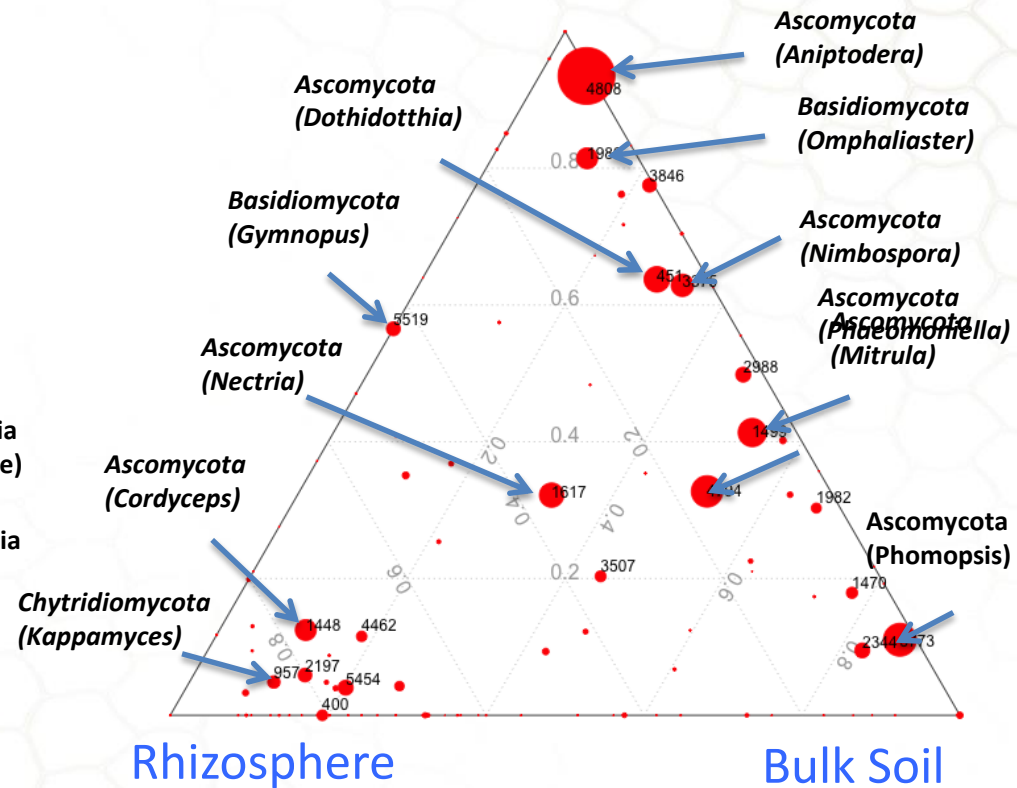
Bacteria

Endosphere



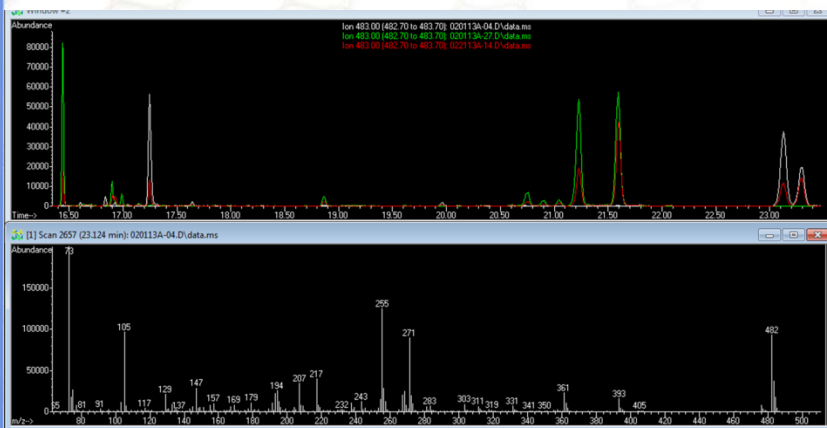
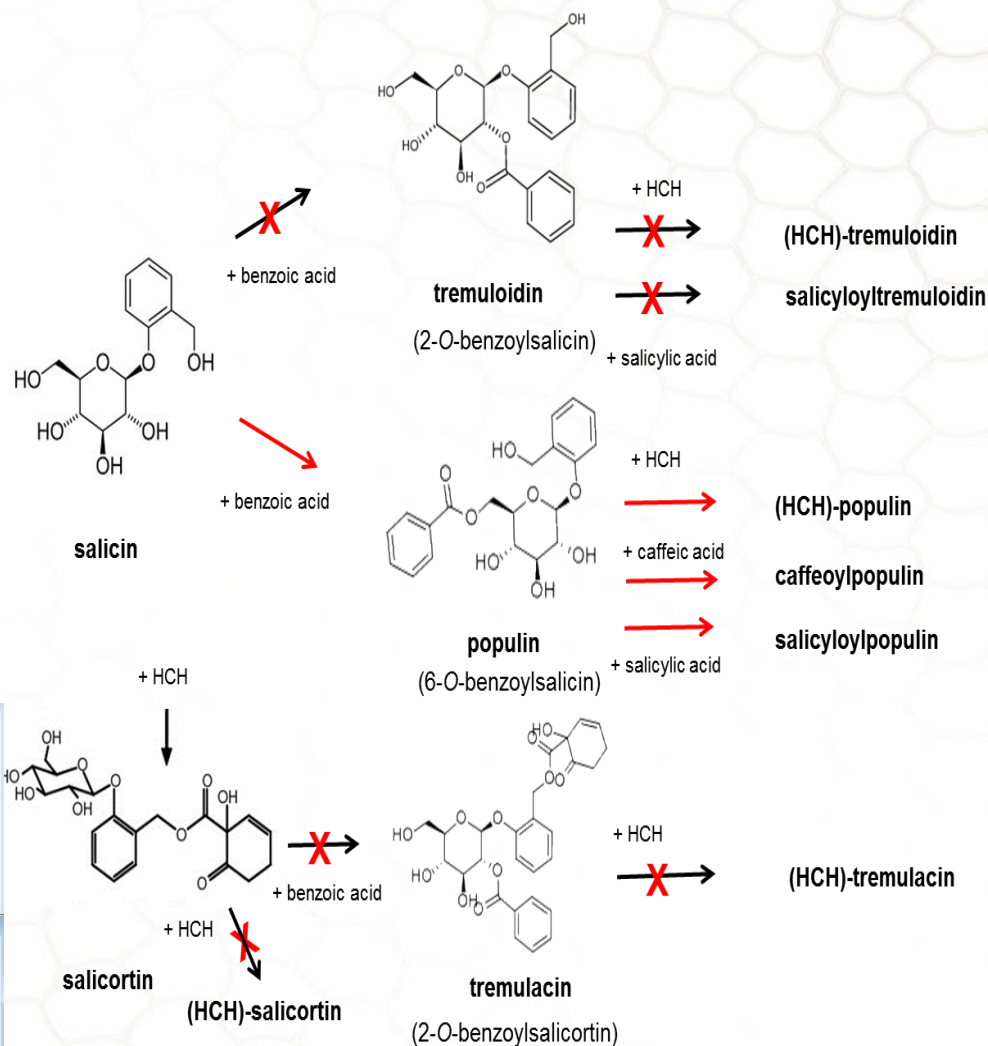
Fungi

Endosphere



Phenolic Content Impacts Endophytes

- Used broad-spectrum GC-MS profiling to identify candidate genes controlling metabolite production
- Developed an algorithm to quantify ~430 peaks
- Identified pathways can be targeted for the over-production of metabolites of interest



Summary and Conclusions

- Photosynthetic assimilation of atmospheric carbon dioxide by land plants offers the underpinnings for terrestrial carbon sequestration.
- A proportion of the C captured in plant biomass is allocated to roots, where it is partitioned into pools of soil organic C and soil inorganic C and can be sequestered for millennia.
- Carbon sequestration can be enhanced through the deliberate use of durable, engineered plant materials and the rational manipulation of its associated microbiome

